

REMARKS

Claims 1-64 are pending. Claims 1-64 have been canceled without prejudice. Claims 65-68 have been added to more specifically point out what applicant regards as the invention. New claims 65 and 66 correspond to previous claim 41, and new claims 67 and 68 correspond to previous claims 42 and 43, respectively. Support for these amendments is found *inter alia* in the specification. Applicant submits that these amendments raise no issue of new matter. Thus, claims 65-68 will be pending and under examination upon entry of this Preliminary Amendment.

Sequence Listing

Applicant submits herewith (a) a paper copy of the sequence listing, inserted into the specification following the Abstract of the Disclosure, (b) a request to use the computer readable form (CRF) of the sequence listing submitted in connection with prior application U.S. Serial No. 09/718,032, attached hereto as **Exhibit A** and (c) a statement in accordance with 37 C.F.R. §1.821(f) attached hereto as **Exhibit B**, certifying that the CRF submitted in the prior application and the written sequence listing contain the same sequence information.

Information Disclosure

In accordance with her duty of disclosure under 37 C.F.R. §1.56, applicant requests that the following disclosures be made of record in the above-identified application pursuant to 37 C.F.R. §1.97(b). These references are also listed on the Form PTO-1449 attached hereto as **Exhibit C**. Copies of items 14, 17, 23, 37, 41, 42, 50, and 51 were previously submitted in connection with U.S. Serial No. 09/082,737, of which the subject application claims priority. Copies of items 1-13, 15, 16, 18-22, 24-36, 38-40, 43-49 and 52-62 were previously submitted in connection with U.S. Serial No. 09/718,032.

1. U.S. Patent No. 5,518,911, Abo, A. et al. issued May 21, 1996;
2. U.S. Patent No. 5,605,825, Abo, A. et al. issued February 25, 1997;
3. U.S. Patent No. 5,698,428, Abo, A. et al. issued December 16, 1997;
4. U.S. Patent No. 5,698,445, Abo, A. et al. issued December 16, 1997;
5. U.S. Patent No. 6,013,464, Abo, A. et al. issued January 11, 2000;
6. U.S. Patent No. 6,013,500, Minden, A. issued January 11, 2000;
7. U.S. Patent No. 6,048,706, Abo, A. et al. issued April 11, 2000;
8. U.S. Serial No. 09/291,417, Plowman, G. et al. published March 13, 2003;
9. Aspenstrom, P. et al. (1996) Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott-Aldrich syndrome. *Curr. Biol.* 6, 70-75;
10. Bagrodia, S. et al. (1995) Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation. *J. Biol. Chem.* 270, 27995-27998;
11. Bashour, A.M. et al. (1997) IQGAP1, a Rac- and Cdc42-binding protein, directly binds and cross-links microfilaments. *J. Cell. Biol.* 137, 1555-1566;
12. Benner, G.E. et al. (1995) Activation of an S6/H4 kinase (PAK 65) from human placenta by intramolecular and intermolecular autophosphorylation. *J. Biol. Chem.* 270, 21121-21128;

13. Bershadsky, A., and Futerman, A. (1994) Disruption of the Golgi apparatus by brefeldin A blocks cell polarization and inhibits directed cell migration. *Proc. Natl. Acad. Sci. U.S.A.* 91, 5686-5689;
14. Brown, J. et al. (1996) Human Ste20 homologue hPAK1 links GTPase to JNK MAP kinase pathway. *Curr. Biol.* 6, 598-605;
15. Burbelo, P.D. et al. (1995) A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases. *J. Biol. Chem.* 270, 29071-29074;
16. Coso, O.A. et al. (1995) The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81, 1137-1146;
17. Cvrckova, F. et al. (1995) Ste20-like protein kinases are required for normal localization of cell growth and for cytokinesis in budding yeast. *Genes Dev.* 9, 1817-1830;
18. Dascher, C., and Balch, W.E. (1994) Dominant inhibitory mutants of ARF1 block endoplasmic reticulum to Golgi transport and trigger disassembly of the Golgi apparatus. *J. Biol. Chem.* 269, 1437-48;
19. Dharmawardhane, S. et al. (1997) Localization of p21-activated kinase 1 (PAK1) to pinocytic vesicles and cortical actin structures in stimulated cells. *J. Cell. Biol.* 138, 1265-1278;
20. Donaldson, J.G. et al. (1992) ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein beta-COP to Golgi membranes. *Proc. Natl. Acad. Sci. U.S.A.* 89, 6408-6412;
21. Donaldson, J.G. et al. (1992) Brefeldin A inhibits golgi membrane-catalysed exchange of guanine nucleotide into ARF protein. *Nature* 360, 350-352;
22. Dutartre, H. et al. (1996) Cytokinesis arrest and redistribution of actin-cytoskeleton regulatory

- components in cells expressing the Rho GTPase CDC42HS. J. Cell. Sci. 109, 367-377;
23. Erickson, J.W. et al. (1996) Mammalian Cdc42 is a brefeldin A-sensitive component of the Golgi apparatus. J. Biol. Chem. 271, 26850-26854;
  24. Erickson, J.W. et al. (1997) Identification of an actin cytoskeletal complex that includes IQGAP and the Cdc42 GTPase. J. Biol. Chem. 272, 24443-24447;
  25. Fukata, M. et al. (1997) Regulation of cross-linking of actin filament by IQGAP1, a target for Cdc42. J. Biol. Chem. 272, 29579-29583;
  26. Hanks, S.K. et al. (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241, 42-52;
  27. Harden, N. et al. (1996) A Drosophila homolog of the Rac- and Cdc42-activated serine/threonine kinase PAK is a potential focal adhesion and focal complex protein that colocalizes with dynamic actin structures. Mol. Cell. Biol. 16, 1896-1908;
  28. Hart, M.J. et al. (1996) IQGAP, a calmodulin-binding protein with a rasGAP-related domain, is a potential effector for cdc4Hs. EMBO J. 15, 2997-3005;
  29. Helms, J.B., and Rothman, J.E. (1992) Inhibition by brefeldin A of a golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. Nature 360, 352-354;
  30. Hillier, L. et al. (1995) yg22e03.r1 Soars infant brain 1NIB Homo sapiens cDNA clone IMAGE:32974 5' similar to SP:KPAK-RAT p35465, EST Database Accession No. R18825;
  31. Johnson, L. et al. (1996) Active and inactive protein kinases: structural basis for regulation. Cell 85, 149-158;

32. Joneson, T. et al. (1996) RAC regulation of actin polymerization and proliferation by a pathway distinct from Jun kinase. *Science* 274, 1374-1376;
33. Kozma, R. et al. (1995) The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss fibroblasts. *Mol. Cell. Biol.* 15, 1942-1952;
34. Kuroda, S. et al. (1996) Identification of IQGAP as a putative target for the small GTPases, Cdc42 and Rac1. *J. Bio. Chem.* 271, 23363-23367;
35. Lamarche, N. et al. (1996) Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP 10 kinase cascade. *Cell* 87, 519-529;
36. Manser, E. et al. (1993) A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42. *Nature* 363, 364-367;
37. Manser, E. et al. (1994) A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature* 367, 40-46;
38. Manser, E. et al. (1997) Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Mol. Cell. Biol.* 17, 1129-1143;
39. Manser, E. et al. (1998) PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. *Mol. Cell.* 1, 183-192;
40. Marshall, C.J. (1994) Signal transduction. Hot lips and phosphorylation of protein kinases. *Nature* 367, 686;
41. Martin, G.A. et al. (1995) A novel serine kinase activated by rac1/CDC42Hs-dependent autophosphorylation is related to PAK65 and STE20. *EMBO J.* 14, 1970-1978;
42. Melnik, M.M. (1997) GenBank Accession No. AF005046;

43. Minden, A. et al. (1994) Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. *Science* 266, 1719-1723;
44. Minden, A. et al. (1995) Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81, 1147-1157;
45. Nobes, C.D., and Hall, A. (1995) Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 81, 53-62;
46. Orci, L. et al. (1991) Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. *Cell* 64, 1183-1195;
47. Pelech, S.L. (1996) Kinase connections on the cellular intranet. *Signalling pathways. Curr. Biol.* 6, 551-554;
48. Rana, A. et al. (1996) The mixed lineage kinase SPRK phosphorylates and activates the stress-activated protein kinase activator, SEK-1. *J. Biol. Chem.* 271, 19025-19028;
49. Schekman, R., and Orci, L. (1996) Coat proteins and vesicle budding. *Science* 271, 1526-1533;
50. Sells, M.A. et al. (1997) Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. *Curr. Biol.* 7, 202-210;
51. Sells, M.A., and Chernof, J. (1997) Emerging from the Pak: the p21-activated protein kinase family. *Trends. Cell. Biol.* 7, 162-167;
52. Sigma catalog, Biochemicals and Organic Compounds for Research and Diagnostic Reagents, "Anonymous" ALA-VAL fragment, pg. 64;
53. Symons, M. et al. (1996) Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. *Cell* 84, 723-734;

54. Szczepanowska, J. et al. (1997) Identification by mass spectrometry of the phosphorylated residue responsible for activation of the catalytic domain of myosin I heavy chain kinase, a member of the PAK/STE20 family. *Proc. Natl. Acad. Sci. U.S.A.* 94, 8503-8508;
55. Takebe, Y. et al. (1988) SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol.* 8, 466-472;
56. Teramoto, H. et al. (1996) Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. A role for mixed lineage kinase 3/protein-tyrosine kinase 1, a novel member of the mixed lineage kinase family. *J. Biol. Chem.* 271, 27225-27228;
57. Van Aelst, L. et al. (1996) Identification of a novel Rac1-interacting protein involved in membrane ruffling. *EMBO J.* 15, 3778-3786;
58. Van Aelst, L., and D'Souza-Schorey, C. (1997) Rho GTPases and signaling networks. *Genes Dev.* 11, 2295-2322;
59. Westwick, J.K. et al. (1997) Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-Independent pathways. *Mol. Cell. Biol.* 17, 1324-1335;
60. Zhang, C. J. et al. (1994) Expression of a dominant allele of human ARF1 inhibits membrane traffic in vivo. *J. Cell. Biol.* 124, 289-300;
61. Zhang, F. et al. (1994) Atomic structure of the MAP kinase ERK2 at 2.3 Å resolution. *Nature* 367, 704-711; and
62. Zhang, S. et al. (1995) Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. *J. Biol. Chem.* 270, 23934-23936.

Applicant: Audrey Minden  
Serial No.: Not Yet Known  
Filed: Herewith  
Page 11

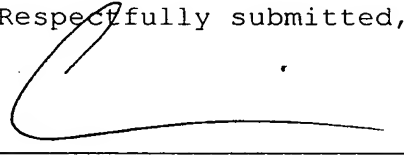
Summary

Applicant maintains that the claims pending in this application are in condition for allowance. Accordingly, allowance is respectfully requested.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the application filing fee, is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



---

John P. White  
Registration No. 28,678  
Alan J. Morrison  
Registration No. 37,399  
Attorneys for Applicants  
Cooper & Dunham, LLP  
1185 Avenue of the Americas  
New York, New York 10036  
(212) 278-0400